D 9 (cont.)

sufficient salt concentration to maintain the hybridization complex, thereby not requiring extraction using reagents such as phenol or chloroform to prepare RNA.

N.E.

20. The method of Claim 19, wherein the biological sample is uncoagulated blood, plasma or bone marrow.

REMARKS

Amended Claims

Claims 1-20 are pending; all claims have been rejected. Claims 1, 3, 6, 9, 18, and 19 have been amended. A marked-up set of claims showing the amendments is attached to this document. No new matter has been added by these amendments. Entry of this amendment and reconsideration of this application is respectfully requested.

Withdrawn Rejections

Applicants note that the previous rejections under 35 U.S.C. § 102 of claims 1-20 have been withdrawn.

Rejections under 35 U.S.C. § 103

A *prima facie* case of obviousness requires one to (1) determine the content and scope of the prior art, (2) ascertain the differences between the prior art and the claims at issue and (3) determine the level of ordinary skill in the art. *Graham v. John Deere Co.*, 383 U.S. 1, 17-18 (1966). For inventions in the biotechnology field, the level of ordinary skill in the art is generally considered to be relatively high (*e.g.*, a Ph.D. or equivalent experience). An obviousness determination also requires consideration of whether the prior art (1) would have suggested to those of ordinary skill in the art that they should make the claimed invention, and (2) would have revealed that, in so doing, those of skill in the art would have had a reasonable expectation of success. *In re Vaeck*, 947 F.2d 488, 20 U.S.P.Q.2d 1438 (Fed. Cir. 1991).

Claims 19-20 have been rejected under 35 U.S.C. § 103(a) over Saunders et al. (U.S. Pat. No. 5,066,792) in view of Barany et al. (U.S. Pat. No. 5,830,711).

Applicants respectfully argue that the invention defined by amended claim 19 and claim 20 is not obvious even if the teachings of Saunders et al. and Barany et al. are combined.

Applicants' method in step b. mixes the biological sample with a solution containing "about 150 mM to about 1M of a soluble salt". In contrast, Saunders et al. teach using a buffer containing 75 mM NaCl and 25 mM Na_2EDTA (see column 5, lines 35-36), and Barany et al. teach using a buffer containing 2 mM EDTA (see column 42, lines 32-36). Neither reference teaches use of a solution containing "about 150 mM to about 1M of a soluble salt" as disclosed by Applicants (see Example 1, beginning at page 23, line 25, particularly at page 26, lines 7-11).

The methods taught by Saunders et al. and Barany et al. both include extraction of nucleic acids from lysates by using reagents that include phenol and chloroform. Saunders et al. teach a method that extracts leukocyte pellets with a buffer combined with phenol, followed by proteinase K digestion, and then additional extractions using phenol, and a mixture of chloroform and isoamyl alcohol (see column 5, lines 34-47). Barany et al. teach a method that includes enzymatic digestions followed by "sequential extractions with phenol, phenol/chloroform, chloroform, [and] n-butanol" (see column 42, lines 40-42). In contrast, Applicants' method is done without extraction using reagents such as phenol and chloroform, as indicated by the amendment to claim 19 and supported by the disclosure at page 16, lines 24-26.

In combining prior art teachings to reject a claim under 35 U.S.C. § 103, there must exist some suggestion, motivation or teaching in the art to combine the references. *In re Rouffet*, 149 F.3 1350, 47 U.S.P.Q.2d 1453, 1457 (Fed. Cir., 1998). To prevent the use of hindsight in an obviousness rejection, an examiner must show a motivation to combine the references that create a case of obviousness, i.e. reasons showing that a skilled artisan, confronted with the same problems as the inventor with no knowledge of the claimed invention, would have selected the elements of the cited prior art for combination in the manner of the applicant's invention. *Id.* at 1457-1458. An examiner cannot be selective in choosing features of the present invention from the prior art teachings to create the claimed combination. *In re Fine*, 837 F.2d 1071, 5 U.S.P.Q.2d 1596 (Fed. Cir. 1988). Also, a reference must be considered for all that it teaches. *W.L. Gore & Associates v. Garlock*, 721 F.2d 1540 at 1550 (Fed. Cir. 1983). Barany et al. teach a method of isolating DNA from a sample that includes the step of *removing* RNA by digestion using RNase A (see column 42, lines 36-38). The Examiner has relied on Barany et al. to teach the substitution of a non-ionic detergent for an ionic detergent as used in the teachings of Saunders et al. The Examiner, however, has ignored the teaching of Barany et al. to *remove* RNA by enzymatic digestion during the DNA

isolation method. Barany et al. thus teach away from the claimed method of preparing RNA.

For all of the above-discussed reasons, a *prima facie* case of obviousness has not been established by combining the teachings of Saunders et al. and Barany et al. Therefore, Applicants respectfully request the withdrawal of the rejections under 35 U.S.C. § 103(a) and allowance of amended claim 19 and claim 20.

Claims 1-18 have been rejected under 35 U.S.C. §103(a), based on the combined disclosures of Eskola et al. (Clin. Biochem., 1994, 27:373-379), Kacian et al. (US Pat. 5,399,491) and Saunders et al. in view of Rowley et al. (US Pat. 5,487,970), Morris et al. (US Pat. 5,529,925), von Lindern et al. (Molec. Cell. Biol., 1992, 12:3346-3355), Goddard et al. (Science, 29 Nov 1991, pp. 1371-1374), Ohki et al. (US Pat. 5,580,727), and Barany et al.

The Examiner has relied on Barany et al. to teach use of non-ionic detergents as in the rejection of claims 19 and 20, discussed above. As discussed above, Barany et al. do not teach use of a solution containing about 150 mM to about 1M of a soluble salt, and do teach enzymatic digestion of RNA and extraction of nucleic acid using organic reagents, including phenol and chloroform. Barany et al. teach away from isolating RNA from a sample. Amended claim 6 makes clear that Applicants' method is performed without extracting the RNA using reagents such as phenol and chloroform, supported by Applicants' disclosure at page 16, lines 24-26. Thus, the teachings of Barany et al. combined with other cited art do not make Applicants' claimed method obvious. Applicants, therefore, respectfully request allowance of amended claim 6.

Independent claims 1 and 9 have been amended by deleting "comprising" and substituting therefor "consisting essentially of". This transition phrase indicates that additional component(s), composition(s) or method step(s) that do not materially change the basic and novel characteristics of the present invention may be included in the compositions or methods of the present invention. Any component(s), composition(s), or method step(s) that have a material effect on the basic and novel characteristics of the present invention would fall outside of this term.

Dependent claims 3 and 6 have been amended consistent with the language of amended claim 1, and dependent claim 18 has been amended consistent with the language of amended claim 9.

Applicants believe that these amendments and arguments fully respond to the Examiner's rejections under 35 U.S.C. § 103(a). Therefore, Applicants respectfully request withdrawal of the rejections and allowance of claims 1-18.

CONCLUSION

In view of the foregoing amendments and remarks, the Applicants respectfully submit that the claims, as amended, are patentable and in condition for allowance. Accordingly, withdrawal of the rejections and allowance of the application is requested. The undersigned has made a good-faith effort to address all the points raised by the Examiner in this Office Action and to place the claims in condition for allowance. If minor matters remain that could be resolved by telephone, the Examiner is invited to contact the Applicants' representative at the number shown below.

Applicants believe there is no fee due in connection with filing this Amendment. If Applicants are incorrect and a fee is required, please debit the appropriate fee from Deposit Account No. 07-0835.

I hereby certify that this correspondence (along with any referred to as being attached or enclosed) is being deposited on the date indicated below with the U.S. Postal Service as First class mail addressed to Box No Fee, Commissioner for Patents, Washington, D.C. 20231.

Respectfully submitted,

Date: May 23, 2001

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Enclosure: Marked-up set of amended claims

Marked Up Copy of Amendments

IN THE CLAIMS:

Kindly amend **Claims 1, 3, 6, 9, 18, and 19** as shown in the following marked-up claims.

1. (Amended 3 times) A method for detecting a fusion nucleic acid [comprising] consisting essentially of the steps of:

a) providing a sample containing a first single-stranded fusion nucleic acid comprising a splice junction;

b) contacting under nucleic acid amplification conditions:

the first single-stranded fusion nucleic acid,

a first primer which hybridizes to the fusion nucleic acid at a first primer binding site located 3' to the splice junction site, and

at least one nucleic acid polymerase activity;

c) amplifying the fusion nucleic acid in an isothermal nucleic acid amplification reaction using the first primer to produce a plurality of second nucleic acid strands complementary to at least a portion of the first single-stranded fusion nucleic acid that contains the splice junction site, wherein each second nucleic acid strand comprises:

a complementary splice junction site,

a first probe binding site located 3' to and not overlapping the complementary splice junction site, and

a second probe binding site located 5' to and not overlapping the complementary splice junction site, wherein the second probe binding site overlaps or is located 3' to sequence complementary to the first primer binding site;

d) hybridizing the second nucleic acid strands with an oligonucleotide probe under hybridization conditions in which the probe hybridizes to either the first probe binding site or the second probe binding site, thereby forming a probe:target hybrid; and

e) detecting the probe:target hybrid as an indication of the presence of the fusion nucleic acid in the sample.

2. (Amended) The method of Claim 1, wherein the first single-stranded fusion nucleic acid is an mRNA, the first primer is a promoter-primer, the polymerase activity comprises an RNA polymerase activity, and the oligonucleotide probe is of the same sense as the mRNA and binds to the first probe binding site.

3. (Amended 2 times) The method of Claim 1, wherein the first single-stranded fusion nucleic acid is a mRNA, wherein the second nucleic acid strands are complementary RNA, [and further compromising] wherein the amplifying step includes contacting the second nucleic acid strand with a second primer or promoter-primer which hybridizes to a second primer binding site located 3' to both the complementary splice junction and the first probe binding site, and wherein the amplifying step uses an RNA polymerase activity, a DNA-directed DNA polymerase activity and an RNA-directed DNA polymerase activity.

4. (Amended) The method of Claim 1, wherein the oligonucleotide probe binds to the second probe binding site and does not form a stable hybridization complex with the first single-stranded fusion nucleic acid.

5. (Amended) The method of Claim 1, wherein the fusion nucleic acid is a *bcr-abl* fusion mRNA and wherein the oligonucleotide probe binds to a *bcr*-derived nucleotide base sequence in the second nucleic acid strands.

6. (Amended 3 times) The method of Claim 1, wherein step a) [further comprises preparing] includes preparing RNA from the sample containing the fusion nucleic acid by:

contacting a biological sample comprising the fusion nucleic acid with a solution consisting essentially of:

a buffer,

about 150 mM to about 1 M of a soluble salt,

about 0.5% to about 1.5% (v/v) of a non-ionic detergent, and

a solid support to which is joined an immobilized oligonucleotide comprising a nucleotide base sequence which forms, directly or indirectly, a stable hybridization complex with an RNA under conditions permitting the formation of the stable hybridization complex; and

separating the hybridization complex joined to the solid support from unhybridized sample components without extracting the RNA using reagents such as phenol or chloroform.

7. The method of Claim 6, wherein the fusion nucleic acid is mRNA.

8. The method of Claim 7, wherein the nucleotide base sequence of the immobilized oligonucleotide comprises a poly-T sequence.

9. (Amended 3 times) A method of detecting a fusion mRNA transcript produced as a result of a chromosomal translocation [comprising] consisting essentially of the steps of:

a) providing a sample containing a fusion mRNA transcript comprising a splice junction;

b) contacting under isothermal nucleic acid amplification conditions:

the fusion mRNA transcript,

a first primer which hybridizes to the fusion mRNA transcript at a first primer binding site derived from a first chromosomal region and located 3' to the splice junction site, and

at least one enzyme having nucleic acid polymerase activity;

c) amplifying the fusion mRNA transcript in a nucleic acid amplification reaction that uses the first primer to produce a plurality of second nucleic acid strands complementary to at least a portion of the

fusion mRNA transcript containing the splice junction site, wherein each second nucleic acid strand comprises:

a complementary splice junction site,

a first probe binding site located 3' to and not overlapping the complementary splice junction site, wherein the first probe binding site is derived from a second chromosomal region, and

a second probe binding site located 5' to and not overlapping the complementary splice junction site, wherein the second probe binding site is derived from a third chromosomal region and overlaps or is located 3' to sequence complementary to the first primer binding site;

d) hybridizing the second nucleic acid strands with an oligonucleotide probe which hybridizes to the second nucleic acid strands at either the first probe binding site or the second probe binding site but does not hybridize to the fusion transcript, thereby forming a hybridization complex of the probe and the second nucleic acid strand; and

e) detecting the hybridization complex as an indication of the presence of the fusion transcript in the sample.

10. (Amended) The method of Claim 9, wherein the amplifying step uses only a first primer that is a promoter primer and the enzyme has an RNA polymerase activity, and wherein the hybridizing step uses an oligonucleotide probe which hybridizes to the second nucleic acid at the first probe binding site.

11. The method of Claim 9, wherein the first probe binding site and the second probe binding site are derived from different locations on the same chromosome in a eukaryotic cell, and the fusion mRNA transcript detected results from an intrachromosomal translocation.

12. The method of Claim 9, wherein the first probe binding site is derived from a different chromosome than the chromosome from which the second probe binding site is derived, and the fusion mRNA transcript

detected results from a translocation involving different chromosomes.

13. The method of Claim 12, wherein the fusion mRNA transcript results from a translocation of human chromosomes selected from the group consisting of: t(1;19), t(2;5), t(2;13), t(4;11), t(6;9), t(8;21), t(9;11), t(9;22), t(11;14), t(11;19), t(11;22), t(12;21), t(14;18) and t(15;17) translocations.

14. The method of Claim 13, wherein the fusion mRNA transcript results from a human t(9;22) translocation and the oligonucleotide probe comprises a *bcr*-derived sequence or an *abl*-derived sequence.

15. One or more oligonucleotides suitable for use in the method of Claim 14, have a sequence selected from the group consisting of SEQ ID NO:1 to SEQ ID NO:23, SEQ ID NO:26 and SEQ ID NO:27.

16. (Amended) The method of Claim 9, wherein the amplifying step uses an RNA polymerase activity, a DNA-directed DNA polymerase activity, and an RNA-directed DNA polymerase activity, and further uses a second primer or promoter primer which hybridizes under amplification conditions to a nucleotide sequence of a complementary RNA produced during the amplifying step.

17. The method of Claim 16, wherein the RNA-directed DNA polymerase activity and DNA-directed DNA polymerase activity are supplied by a reverse transcriptase.

18. (Amended 2 times) The method of Claim 9, [further comprising the steps of amplifying] <u>wherein the amplifying step also amplifies</u> an internal control transcript in the sample by using the first primer and then hybridizing a second oligonucleotide probe which hybridizes to the complement of the internal control transcript but does not hybridize to the complement of the fusion mRNA transcript thereby forming in internal control hybridization complex, and [detecting] <u>wherein the detecting step also detects</u> the presence of the internal control hybridization complex in the sample, thereby providing an internal standard.

19. (Amended 3 times) A method of preparing a sample containing RNA suitable for amplification, comprising the steps of:

a) providing a biological sample comprising unpurified RNA;

b) mixing the biological sample with a solution consisting essentially of:

a buffer at a pH of about 6.5 to about 8.5,

about 150 mM to about 1M of a soluble salt,

about 0.5% to about 1.5% (v/v) of a non-ionic detergent, and with

a solid support to which is joined an immobilized oligonucleotide comprising a nucleotide base sequence which forms a stable immobilized oligonucleotide:RNA hybridization complex under hybridization conditions;

c) separating the hybridization complex joined to the solid support from unhybridized sample components; and

d) then washing the hybridization complex joined to the solid support with a solution having sufficient salt concentration to maintain the hybridization complex, thereby not requiring extraction using reagents such as phenol or chloroform to prepare RNA.

20. The method of Claim 19, wherein the biological sample is uncoagulated blood, plasma or bone marrow.